THE METHOD OF SECTION-CUTTING WITH SOME IMPROVEMENTS.

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It is a well-known fact that the structure of many organs of plants and animals cannot be investigated without getting a direct insight into the interior. It is easy enough to place a small portion of the interior of any organ under the microscope, but it is impossible in that way to ascertain the mutual position of parts. To enable the observer to study them under the microscope and in sufficient connection with other parts, so that their position can be defined, there is only one method, that of section cutting.

Organs of plants and a few hard parts of animals can be cut as they are. It is advantageous to place such in a slit made in a cork and cut them together with the adjacent cork with a sharp razor. In cutting sections of plants, which are damp, it is necessary to wet the razor so that the section, when cut, can immediately be immersed in water and so prevented from drying and shriveling up.

Soft animal parts must be hardened. Any one of the methods described in my paper on the preservation of tender marine animals (proceedings of the Linnean Society of New South Wales, Vol. IX.) can be used. The result will in every case be that the hardened organ will be preserved in strong spirits, with which it will also be saturated. The other methods of cutting with the hand, and hardening and embedding simultaneously, and the freezing method, do not seem to yield such good results as the one to be described below.

Brothers Hertwig (Das Nervensystem und die Sinnesorgane der Medusen) have made use of the former of these two methods. They placed the specimen, after it had been treated with osmic acid and stained with alum-carmin in a solution of gum-glycerine,

and enclosed it between bits of fresh liver. The whole was then placed in strong spirits, where the liver, together with the gum-glycerine, got very hard, and enclosed the specimen which was afterwards cut by hand. This method is an old one, and has often been used before for less tender animals than those, to which the brothers Hertwig have applied it, namely, the small jellyfish.

Sollas (Sponge Fauna of Norway, Annals and Magazine of Natural History, 1879) recommends the freezing Microtome. The specimen is placed in glycerine-gelatine and hardened, not by the influence of spirits of wine but by cold, which is produced by a small refrigerating apparatus. The specimens treated in this way are cut by a machine or Microtome.

Both the sections cut in liver and with the freezing Microtome cannot be placed in Canada balsam but must be kept in glycerine or gelatine and are consequently liable to attain too great a transparency in course of time and also to get dim in consequence of the growth of Bacteria under the cover glass.

The method which is recognised as the very best by most microscopists is the one which I have been using and improving for several years, and a description of which follows.

The Specimen.

It is of great importance that as small a piece as possible should be cut out of the organ, that all unnecessary parts should be cut away and the whole thing cut into such a shape that it will afterwards be easy to find out what position the specimen had in the organ from which it has been taken.

Hardening.

To small and tender organs *osmic acid* can be recommended as yielding better results than any other re-agent known. The specimen is placed in a solution varying in strength from 0·5—2% and left there for a short time. Both time and strength depend on the nature of the organ which is to be cut.

Corrosive sublimate, a concentrated solution to be used, which may be made stronger by warming. The specimen, if tender,

should be dipped in the solution if warm and left a short time if cold. Tough organs can remain for some time in the solution. Wilson (On Alcyonarians, Mittheilungen der zoologischen Station in Neapel, 1884,) recommends to leave soft corals for several hours in a warm concentrated solution of corrosive sublimate. For most purposes this will be too strong.

Picric acid solution or a mixture of it with sulphuric acid diluted has been used by many with good results, so particularly Kleinenberg (Hydra). It is particularly good for staining nervous fibres which are coloured yellow by it.

Chromic acid or Bichromate of potassium in dilute solution is used for brains to great advantage.

Chloride of gold 1% solution colours nerve fibres and tissue cells and the intercellular substance.

Nitrate of silver is reduced by the intercellular substance and eventually turns it black so that the limits of the cells can be made clearly visible.

Chloride of Palladium hardens the tissue and colours, muscular fibre brown. Cloride of iron has also been recommended for hardening the tissue.

Most metallic salts have some influence or other on the Protoplasm, and in every case the metal enters into combination with it, changing its colour and making it hard.

A specimen treated with one of these re-agents will stand washing in distilled water without any perceptible change taking place.

Staining in Toto.

The specimens should be stained, because the colour not only makes certain parts more distinct, but also counteracts the noxious after effects of the hardening re-agents. Osmic acid, gold, and other specimens, invariably commence to darken after some months if they have not been stained.

The specimens can either be stained when whole, or the sections can be coloured. The staining in toto saves much trouble and time, and is also advantageous, because the sections have not to be

handled so much. On the other hand the staining in toto has two great drawbacks. Firstly, it very often happens that with every care the colour does not penetrate to the centre of the specimen, and even if this result has been attained it is very difficult to stain the whole specimen in a uniform manner; the outer portions will generally be stained in a greater degree than the inner ones. Secondly, it is necessary to keep the specimen in the colouring solution for a long time to attain thorough staining, and it is very difficult with most colouring liquids to prevent maceration from spoiling the whole specimen. Even if the specimen is kept on ice the epithelia will often be loosened and the whole thing spoilt if the specimen is left in the staining solution over night.

In a hot country such as this it is difficult to prevent this.

Any colour in solution will do for staining, and a wide scope is left to the microscopist in this field.

Aniline dyes have been extensively used of late, but although they are of great help to those who study bacteria, they have not yielded any good results with higher organisms. Eosin, Fuchsin, Bismarckbrown, &c., have been tried. They stain very rapidly, and diffuse through the tissue with greater rapidity than any other colouring fluid. The fault they have is, that they stain everything in the same way and nearly in the same degree, so that one sees in a section stained with aniline dye hardly more than in a section not stained at all.

Carmine, in different solutions, is a very much better colour. It stains the "chromatin" of the protoplasm very much, and all the other parts of the tissue only faintly. We know in what kind of cells and parts thereof this chromatin is contained, and we can therefore attain a clearer insight into the structure of any specimen by colouring with carmine, than we could with the investigation of not coloured sections. The carmine can be dissolved in various ways—I refer the reader to text-books on Histology, mainly Renvier—the solutions I have found best are those in alum and picric acid. Carmine powder is dissolved in a concentrated solution of alum when still hot and immediately

filtered ; a deep red solution passes through and can be used as it is, or for very tender specimens diluted with 50 % of destilled water. This alum-carmine stains the nuclei of all cells ; the ova spermatozoa and ganglia cells, and also muscular and gland cells very intensely, whilst it gives to the ordinary connective tissue only a slight red tinge.

Picric-acid-carmine is a most excellent colouring fluid for the tissue of higher animals. It stains like the foregoing, the nuclei, ganglea cells, &c., red, but the picric acid in it at the same time colours the horny substance of the skin and also nerve fibres intensely orange-yellow.

Hæmotoxylin is also an excellent staining fluid, prepared according to the Kleinenberg. It stains the ordinary protoplasm light violet and the nuclei dark violet, nearly black.

I refer the reader who may wish to collect some further particulars on this subject to the standard works on Histology by Renvier and Klein.

Preparation.

After the specimen is coloured it must be well-washed so as to remove all colouring matter, which has not entered the protoplasm or other parts of the specimen in a fixed combination.

Cold water will in most cases be the best thing. For Haematoxylin, spirits.

When the specimen is washed it can either be imbedded in gum Glycerine, frozen and cut forthwith or imbedded in gum glycerine enclosed in liver, hardened in spirits and cut with the hand as described above, or also prepared for imbedding in paraffin.

I, together with many other zoologists, consider the paraffin imbedding method to be far superior to any other.

The first thing to be done with the washed specimen to prepare it for imbedding in paraffin, is to extract all the water from it by means of alcohol. This is a very difficult operation in the case of very tender specimens, and requires great patience and care.

If the specimen is placed in strong spirits immediately it will shrink and become totally useless, because the water is extracted from it by the strong hygroscopic spirits with such great violence.

It is therefore necessary to place the specimen in a series of mixtures of alcohol and water, getting continuously stronger until it is placed in pure absolute alcohol.

It is always connected with difficulties to procure this latter, wherefore I recommend it to the workers in the colonies to make it themselves. Ordinary strong *not methylated* spirits of wine are mixed with quick lime, which will absorb all the water, and the pure absolute alcohol can be distilled from this mixture of spirits and quicklime. In this way the spirits which have been used can always be utilised again, and the whole process will save a great deal of expense in the course of time.

Good non-methylated spirits of wine must be procured, and its percentage of alcohol ascertained. The spirits of wine consists of a mixture of water and absolute alcohol ; the percentage of which is expressed in a most unpractical and clumsy way by the term x over proof. By means of an areometer it is easy to ascertain the percentage of alcohol in the spirits. This mixture can then be mixed with absolute alcohol or water, and any desired strength produced.

I can recommend the following mixtures for practical use, 30%, 50%, 70%, 90%, 100% of Alchohol in 100 parts of the mixture.

The specimen, when washed is firstly placed in 30% alchohol, left there several hours, then in 50% left there for some hours, then 70%, 90% and finally 100% or absolute alcohol and left in each mixture 2-4 hours. In the absolute alcohol, which ought to be changed once or twice, 12 hours to a day.

In this way it is possible to get all the moisture out of the tenderest specimen without it shrinking.

The specimen is then placed for a few hours in a solution of alcohol in ether and finally in ether, where it again should remain for a day. The specimen will lie at the bottom. Chloroform is then poured into the ether (Giesbrecht) and being much heavier than the latter it sinks down to the bottom, whilst the ether swims on the surface. The specimen then lies between the two, below the ether and above the chloroform. The ether contained in the

specimen is slowly exchanged for chloroform and, finally, when nearly all the ether is replaced, the specimen sinks down to the bottom. It can then be removed and placed in fresh pure chloro form and left there for 12 hours.

It is now ready for imbedding.

The Imbedding in Paraffin.

The imbedding is also a process which requires much care, patience and experience. If the beginner often loses his specimen in consequence of the want of the latter, he need not be disheartened or ashamed; this happens repeatedly to every one, and I have seen even men like Lang and F. E. Schulze spoil their specimens in imbedding.

I have found it advantageous to place the specimen for a day or two in molten soft paraffin and keep the latter liquid and at the uniform temperature of 47—49° C., in a self-regulating water-bath stove. During this time all the chloroform has escaped and the specimen can be placed in another little cup with molten hard paraffin kept at a temperature not exceeding 58° C. Pretty hard paraffin will melt at that point. It can be left there for an hour or so and can then be imbedded. A trough of paper or brass measuring $1\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2}$ inches is filled with molten hard paraffin and at the time when it commences to solidify the specimen is placed in it. The "freezing" surface is kept open by heated needles and the specimen is put in position by the same implements

It is of the greatest importance to put the specimen in such a position that the desired direction of the sections can be obtained by cutting the paraffin stick, cast in the trough straight across that is vertical to its longitudinal axis. This is often a matter of great difficulty, and it will always be of advantage to throw a ray of reflected and concentrated sunlight into the molten paraffin, so as to illuminate the specimen properly. It is of course most convenient to cut to an elongate shape before in such a manner that one can tell the position in which it ought to be imbedded by the outer shape of the specimen.

Cutting.

The paraffin block should cool down slowly, and be made as cold as possible before cutting. The colder the paraffin the harder, and the harder the better.

Caldwell recommended a very ingenous method of cutting sections which has been adopted in several places, but which is in practice by no means so beautiful as in theory.

He encloses the hard paraffin with a thin coating of soft paraffin, and in cutting, the one section sticks to the *opposite margin* of the other. If cutting is continued a "ribbon" of sections is produced which can be caught up by a moving bit of tape, and portions of which can be cut out showing continuous series of sections.

The Paraffin block can be either cut by the hand or placed in a section-cutting apparatus termed a Microtome, and cut either by hand or automatically.

Cutting with the hand has been nearly universally abandoned (Leuckart), and the Microtomes are used for the purpose. The principal always is, that the knife moves backwards and forwards whilst the paraffin block is slowly raised. Or the knife is steady and the paraffin block rises and moves backwards and forwards at the same time. (Caldwell.) In the simpler instruments, one moves the holder of the knife backwards and forwards in a metal groove with the right hand, and raises the specimen to be cut, with the left either by pushing it up along an inclined groove. (Leiser), or by turning a screw (the common form.)

Automatic Microtomes are made by the Cambridge Mechanics' Institution, which can be worked by water power or by treading, and the model has been copied by many with diverse alterations. These automatic instruments yield very excellent results as long as they are in order, but being so complicated they are apt to get out of order. These are all adapted for the ribbon method and sections of any desired thickness that is to the limit at which the sharpest razor at a certain angle will cut can be made in continuous series of specimens, measuring two inches across.

To all these instruments the same remarks apply, and everywhere the same difficulties are encountered.

The success of making good sections depends firstly on the quality and secondly on the position of the knife. No expense should be spared to obtain the best razor possible for the purpose. The knives, which are sold specially for the purpose, are always bad and utterly useless.

The razor can be fixed either at right angles to the direction in which the knife is moved, or obliquely. The opinions as to which is the best differ. Caldwell and I use the knife vertical to the direction of the motion. Further the plane of the knife itself can be altered so that the cutting edge dips more or less downward. With an angle of about 12° to the plain in which the knife moves the finest sections can be cut. The difficulty of having the knife so steep is that then the sections tend to curl up.

This latter is a great evil and not altogether overcome by the appliance of an outer carting of soft paraffine as recommended by Caldwell.

F. E. Schulze has in recognition of this difficulty of the curling up of the sections constructed an apparatus which prevents it to a great extent, so much so, that if Schulze's method is combined with Caldwell's one can be pretty sure of obtaining good sections.

A smooth round rod is fixed to the knife in such a manner, that it lies parallel to, and just above the cutting edge so that any section cut must pass through between the blade of the razor and this rod, which can be attached by springs or screws to the back of the knife.

I would recommend therefore that such an additional apparatus be added to any of the Microtomes in trade.

The outer coating of soft paraffin can be produced in a most simple manner.

When the paraffin is cold and hard, all the unnecessary parts around the specimen are cut away with a knife so that only a millimetre of paraffin is left on every side of it. The top is then removed. The lower end, which is subsequently fixed in the paraffin-holder of the Microtome should be cut down just to fit into the holder.

When this has been done the top of the paraffin-stick containing the specimen is dipped into a freezing molten mass of soft paraffin and instantly withdrawn.

The desired coating of soft paraffin will then be found on the outer surface.

There should always be *continuous series* of sections cut and mounted, one after the other. For certain things, and particularly for a preliminary investigation, this is not necessary to such an extent, and it will save time, trouble and material, if in such a case every second section is cut thick and thrown away, and every second cut to the required fineness and mounted.

The thickness of sections is a point on which a great deal depends. The mutual position of whole organs or groups of cells can generally be ascertained much better by means of thick sections and low powers, than by means of the very fine sections.

For histological detail a section is rarely too fine. The thickness required varies with every case, and the thickness to choose must be left to the investigator and his experience.

For an investigation into the structure of a rare and valuable specimen, a continuous series of sections might perhaps be recommended, which are alternately very thin—as thin as they can be made—and of medium thickness, perhaps 0·005—0·02 mm.

Mounting.

The sections when attached to one another as a ribbon, can be placed on a slide by cutting a portion of the ribbon corresponding in length to the size of the slide off, and placing it there. Otherwise it is necessary to place the sections as they are made, one after the other on the slide. It is hereby very important, that they all should be parallel to one another.

Before placing them on the slide, the latter must receive a coating of shellac. White shellac is dissolved in absolute alcohol and allowed to stand. A sediment will form and the clear solution can be poured off. This is put on to the slide with a brush just before use.

The sections are placed on the slide as soon as the shellac film is dry and the whole slide then placed in the water-bath stove, where it is just so much heated as necessary to soften and partially melt the paraffin and to soften the shellac, about 55°—60° C. As soon as the paraffin has melted, the slide is immersed in a flat dish, filled with spirits of turpentine. The sections adhere in consequence of the shellac to the slide, the turpentine dissolves and removes the paraffin. The slide can be taken out of the turpenine bath at convenience, but with great care so as not to create a strong current of turpentine on its surface, which might wash some of the sections off and so spoil the series.

If staining is not required, the sections are now ready for mounting in Canada balsam. The best way is to drain off the turpentine by holding the slide upright and wiping the margin, then to put drops of a Canada balsam or Damar resin on the sections and finally to cover with the cover glass.

Caldwell and others use slides 6 x 2, with cover glasses of exceeding thinness and nearly equal size, which I can warmly recommend.

The ordinary cover glasses on sale here, are much too small and much too thick for any fine work.

The Canada balsam or Damar resin, the latter is to be preferred on account of its white colour, should be dried in the oven but not heated above 75° to prevent the Canada balsam from turning brown, which it invariably does if heated too much. When dry, brittle and hard, the resin or Canada balsam, is dissolved in chloroform. A mixture of 50 % Canada balsam or Damar and 50 % chlololform has the most convenient consistence, and will get hard in an hour on the outer margin and never cracks.

The mounted sections should be left until the free margin of the resin has consolidated and then they can be examined.

As a hint to beginners, I would add, never omit to let the sections pass under a low power both at the beginning and at the termination of the examination.

Staining the Sections.

I have alluded above to the difficulties connected with staining in toto, and will now dwell on the staining of the sections. They are of course made just in the same way as described above. The staining process is omitted. The specimen is placed in the weakest alcohol, as soon as the hardening re-agent has been washed out. The further process as above until the sections on the slide are taken out of the turpentine bath. Haswell uses colours mixed with turpentine or similar oils for staining his sections an equally simple as commendable method. The slides are removed from the turpentine bath and placed in a solution of the desired colour in turpentine or any oil. Any transparent oil-colour can be used for the purpose. Carmine yields also here very good results.

The ordinary staining-reagents can also be used, but this requires a complicated and to the sections injurious process of washing. The turpentine must be washed out with absolute alcohol. This must be replaced by spirits of wine and finally water. Then the sections are stained, after which they must be washed, and treated with increasing strengths of alcohol as before, finally they must be covered with clove oil and then mounted in resin as above. If the staining is performed with an oil-colour, the sections are immersed for a few minutes in the colouring matter, and then replaced in the turpentine bath and afterwards mounted as above.